Thermodynamics of the Base-On/Base-Off Equilibrium of Alkyl-13-epi- and Alkyl-8-epicobalamins: Understanding the Thermodynamics of Axial Ligand Substitution in Alkylcobalt Corrinoids

Kenneth L. Brown* and **Guo-Zhang Wu**

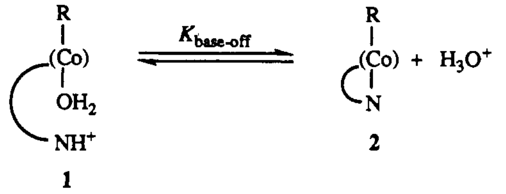
Department of Chemistry, Box CH, Mississippi State University, Mississippi State, Mississippi 39762

Received September 16, 1993®

The apparent pK_a's for the base-on/base-off reaction of cobalamins have been measured as a function of temperature for cyanocobalamin (CNCbl), a series of seven alkyl-13-epicobalamins (R-13-epiCbl; R = CH_3CH_2, $NC(CH_2)_3$, CH_3, CF_3CH_2, $NCCH_2$, CF_3, CN), in which the *e* propionamide side chain is epimerized from the lower to the upper corrin ring face, and a series of four alkyl-8-epicobalamins (R-8-epiCbl; R = CH_3CH_2, CF_3CH_2, CF_3, CN), in which the *d* propionamide side chain is epimerized from the lower to the upper corrin ring face. These values have been used to calculate the equilibrium constant, K_{Co}, for the intramolecular axial nucleotide off/on equilibrium and the enthalpy and entropy change for this reaction. Taken together with previous results for a series of nine alkylcobalamins (RCbl), the data show that the free energy change and enthalpy change for this ligand substitution equilibrium are slightly sensitive to epimerization of the *e* and *d* propionamide side chains. However, the entropy change is independent of the Cbl epimer for a given R group. This suggests that changes in corrin side chain thermal motions accompanying the base-off/base-on transition do not contribute significantly to the entropy change for this reaction, which is, instead, dominated by changes in axial ligation. Since $-\Delta S_{Co}$ decreases substantially across each series of complexes as the organic ligand becomes increasingly electron withdrawing (and $-\Delta G_{Co}$ increases), it seems highly unlikely that the reaction represents the conversion of a hexacoordinate base-off aqua complex to a hexacoordinate base-on complex for all R. These results consequently provide thermodynamic evidence that the base-off RCbl's exist as an equilibrium mixture of hexacoordinate aqua species and pentacoordinate species, the position of the equilibrium being sensitive to the nature of the organic ligand.

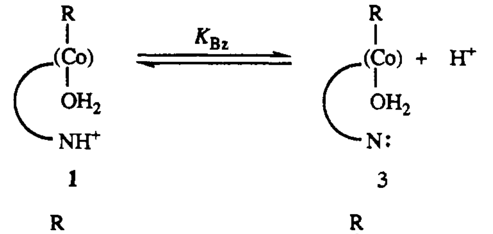
Introduction

There has long been interest in the so-called base-on/base-off reaction of cobalamins (Cbl's;[1] Figure 1), in which the axial nucleotide is uncoordinated and protonated in acidic aqueous solution (eq 1).[2-13] Dubbed the red–yellow shift[2] due to the large change in the UV–visible spectrum accompanying this equilibrium, it represents an intrinsic chemical property so basic to Cbl's that its thorough understanding would seem to be a prerequisite for comprehending the chemistry of these interesting compounds. Because of the large spectral changes accompanying this reaction, highly precise measurements of p$K_{base\text{-}off}$ can readily be made. Values have been reported spanning the range of 5.18 (for $(CH_3)_3CCH_2Cbl$[14]) to −2.13 (for H_2OCbl^+[15]), testimony to the importance of the trans influence of the upper axial ligand on the affinity of the pendent Bzm[1] nucleotide for the metal atom.

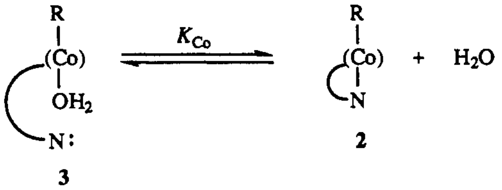


(1)

Since the acid–base chemistry of the detached axial nucleoside (α-ribazole[1]) and nucleotide (α-ribazole 3′-phosphate[1]) of Cbl's is well-known,[10,11,16] the composite equilibrium of eq 1 can be factored into its component parts (eqs 2 and 3), and values for the equilibrium constant for the intramolecular ligand substitution



(2)



(3)

® Abstract published in *Advance ACS Abstracts,* July 15, 1994.

(1) Abbreviations: Cbl, cobalamin; RCbl, alkylcobalamin; R-8-epiCbl, alkyl-8-epicobalamin; R-13-epiCbl, alkyl-13-epicobalamin; AdoCbl, 5′-deoxyadenosylcobalamin (coenzyme B_{12}); $RCbi^+$, alkylcobinamide; Bzm, 5,6-dimethylbenzimidazole; α-ribazole, 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole; α-ribazole 3′-phosphate, 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole 3′-phosphate; cobaloxime, bis(dimethylglyoximato)cobalt; Saloph, dianion of disalicylidene-*o*-phenylenediamine; BAE, dianion of bis(acetylacetone) ethylenediimine.

(2) Ladd, J. N.; Hogenkamp, H. P. C.; Barker, H. A. *J. Biol. Chem.* **1961**, *236*, 2114.

(3) Dolphin, D. H.; Johnson, A. W.; Rodrigo, R.; Shaw, N. *Pure Appl. Chem.* **1963**, *7*, 539.

(4) Hogenkamp, H. P. C.; Oikawa, T. G. *J. Biol. Chem.* **1964**, *239*, 1911.

(5) Hayward, G. C.; Hill, H. A. O.; Pratt, J. M.; Vanston, N. J.; Williams, R. J. P. *J. Chem. Soc.* **1965**, 6485.

(6) Hogenkamp, H. P. C.; Rush, J. E.; Swenson, C. A. *J. Biol. Chem.* **1965**, *240*, 3641.

(7) Pratt, J. M.; Thorp, R. G. *J. Chem. Soc. A* **1966**, 187.

(8) Pratt, J. M. *Inorganic Chemistry of Vitamin B_{12}*; Academic Press: London, 1972.

(9) Chemaly, S. M; Pratt J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2267.

(10) Brown, K. L.; Hakimi, J. M.; Nuss, D. M.; Montejano, Y. D.; Jacobsen, D. W. *Inorg. Chem.* **1984**, *23*, 1463.

(11) Brown, K. L. *J. Am. Chem. Soc.* **1987**, *109*, 2277.

(12) Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3548.

(13) Brown, K. L.; Satyanarayana, S. *Inorg. Chim. Acta* **1992**, *201*, 113.

(14) Brown, K. L. Brooks, H. B. *Inorg. Chem.* **1991**, *30*, 3420.

(15) Brown, K. L.; Hakimi, J. M.; Jacobsen, D. W. *J. Am. Chem. Soc.* **1984**, *106*, 7894.

(16) Brown, K. L.; Hakimi, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 496.

0020-1669/94/1333-4122$04.50/0

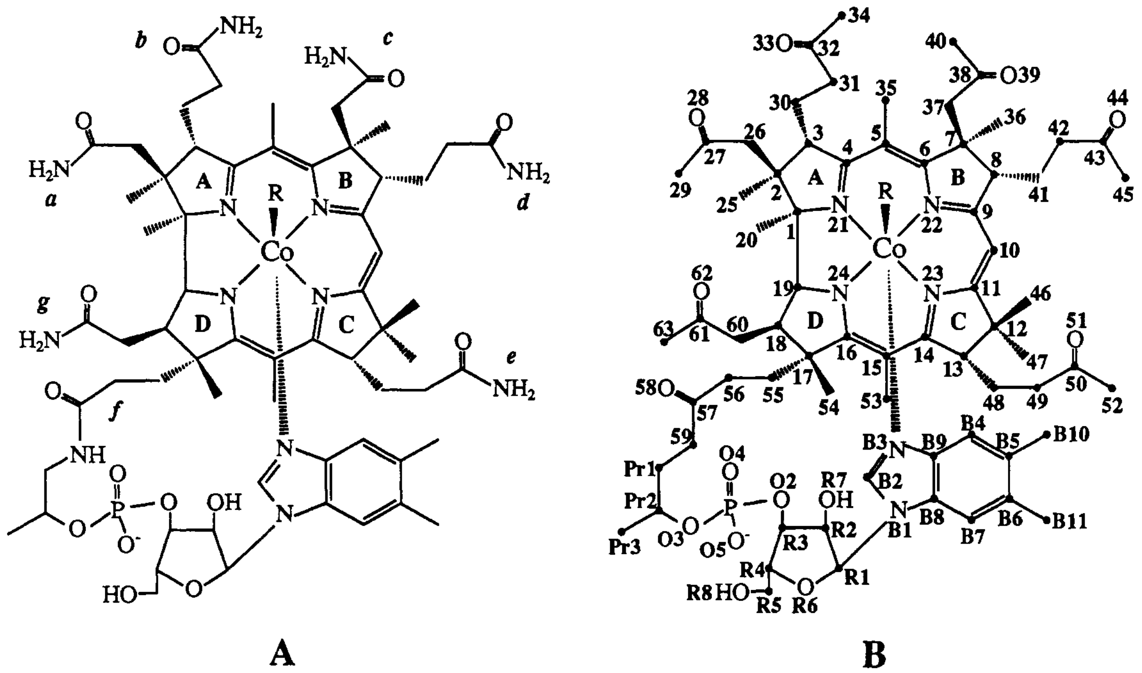


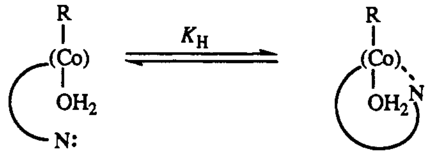
Figure 1. (A) Structure and (B) numbering scheme of an alkylcobalamin (RCbl). In the R-13-epiCbl's the *e* propionamide side chain projects above the corrin ring plane, and in the R-8-epiCbl's the *d* propionamide side chain is equatorial.

reaction (K_{Co} in eq 3) can be calculated from eq 4.[17] The latter

$$K_{Co} = (K_{base\text{-}off}/K_{Bz}) - 1 \qquad (4)$$

equilibrium has been shown to be ionic strength independent, and values of K_{Co} can vary by over 7 orders of magnitude, from 0.75[14] to 4.9 × 10^7.[12,15]

An extremely careful study of the thermodynamics of eq 3 for CH_3Cbl and $^-OOCCH_2Cbl$ by ^{13}C NMR[12] has revealed the presence of an additional species in that reaction (eq 5). This



3 4 (5)

species (**4**), referred to as the "tuck-in" species of unprotonated base-off cobalamin, has been characterized by ^{13}C and ^{15}N NMR[21,22] as an intramolecular complex in which the Bzm nucleotide is associated with the *g* acetamide side chain via a hydrogen bond between the B3 nucleotide nitrogen and the *g* amide (Figure 1). As anticipated for formation of a species stabilized primarily by a hydrogen bond in water, K_H (eq 5) is temperature independent, the interaction being driven entirely by entropy.[12,23] Thus, the correct equation for calculating K_{Co} (eq 3) from $K_{base\text{-}off}$ (eq 1) is eq 6. However, since all of the known values of K_H are quite small ($K_H < 4.2$),[12,23] inclusion of the K_H term only significantly affects the calculation of K_{Co} if $K_{base\text{-}off}/K_{Bz} < 100$ (i.e., $pK_{base\text{-}off} < 3.5$ at 25 °C).

$$K_{Co} = (K_{base\text{-}off}/K_{Bz}) - K_H - 1 \qquad (6)$$

Studies of the temperature dependence of K_{Co}[10,12] have revealed the surprising fact that, for a series of eight RCbl's (R = CH_3CH_2, $CH_3CH_2CH_2$, $NCCH_2CH_2CH_2$, $CH_3OCH_2CH_2$, CH_3, CF_3CH_2, CF_2H, CF_3) for which the value of K_{Co} varies by nearly 3 orders of magnitude, the enthalpy change associated with eq 3 is invariant ($\Delta H_{Co} = -7.8 \pm 0.6$ kcal mol^{-1}). Thus, all of the variability in K_{Co}, and consequently in $pK_{base\text{-}off}$ (which varies from 4.16 to 1.44 across this series), is due to the entropy change, ΔS_{Co}, which varies from ca. −24 to ca −5 cal mol^{-1} K^{-1}. The constancy of ΔH_{Co} could conceivably be attributed to identical sensitivities of the Co–OH_2 and Co–N_{Bzm} bond enthalpies to changes in the trans axial ligand, although such an explanation seems rather unlikely. The strong trend in the ΔS_{Co} values has, however, never been explained.[10,12]

The importance of the entropy term, ΔS_{Co}, in the variation of K_{Co} with R suggests that our current picture of the base-on/base-off reaction is incomplete. One possibility is that a 5-coordinate intermediate may intervene in eq 3 and that the position of the 5-coordinate/6-coordinate equilibrium is significantly dependent on R. The only other possibility would seem to be the involvement of corrin ring side chain thermal mobility in the base-on/base-off reaction, since these side chains represent the only regions of these complexes with sufficient mobility to provide the observed entropy changes.[24] Indeed, there is evidence that the *d* and *e* propionamide side chains may well interact with the axial nucleotide. An examination[26] of the X-ray crystal structure of CNCbl[27] with hydrogen atoms placed in standard positions using the molecule graphics program Alchemy III shows

---

(17) Substantial information of the thermodynamics of the equilibrium of eq 3 in ethylene glycol for several RCbl's has also been obtained by Finke and co-workers.[18-20] However, as there is evidently a large solvent effect on this equilibrium, these results are not directly relevant to the current work in aqueous solution.

(18) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469.

(19) Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 585.

(20) Waddington, M. D.; Finke, R. G. *J. Am. Chem. Soc.* **1993**, *115*, 4629.

(21) Brown, K. L.; Brooks. H. B.; Zou, X.; Victor, M.; Ray, A.; Timkovich, R. *Inorg. Chem.* **1990**, *29*, 4841.

(22) Brown, K. L.; Brooks, H. B.; Gupta, B. D.; Victor, M.; Marques, H. M.; Scooby, D. C.; Goux, W. J.; Timkovich, R. *Inorg. Chem.* **1991**, *30*, 3430.

(23) Brown, K. L.; Evans, D. R. *Inorg. Chim. Acta* **1992**, *197*, 101.

(24) Recent studies of the energetics of the thermal homolysis of RCbl's with bulky organic ligands (i.e., $C_6H_5CH_2Cbl$ and $(CH_3)_3CCH_2Cbl$)[14,25] have pointed to the importance of corrin ring side chain thermal motions in the entropic activation of such RCbl's for Co–C bond homolysis. Thus, the bulky organic ligand is seen as interfering with the rotational freedom of motion of the upward projecting *a*, *c*, and *g* acetamide side chains (Figure 1) in the ground state. Release (or reduction) of this interference as the organic radical and cob(II)alamin separate in the transition state then provides an entropic drive for thermolysis.

(25) Brown, K. L.; Brooks, H. B.; Behnke, D.; Jacobsen, D. W. *J. Biol. Chem.* **1991**, *266*, 6737.

(26) Marques, H. M.; Brown, K. L. Manuscript in preparation.

(27) Brink-Shoemaker, C.; Cruickshank, D. W. J.; Hodgkin, D. C.; Kamper, M. J.; Pilling, D. *Proc. R. Soc. London, A* **1964**, *278*, 1.

that the H atom on B2 is within 2.3 and 2.4 Å, respectively, of the two H atoms on C48 and that the H atom on R5 is within 2.7 Å of the H atom on C49. The *d* side chain is virtually parallel to the plane of the Bzm rings, and the closest contacts occur between a H atom on C41 and B4H (2.3 Å), B4 (2.6 Å), and B5 (3.0 Å).

Any hypothesis regarding the involvement of side chains in the entropy change associated with eq 3 is directly testable by studies of RCbl analogs in which the corrin ring side chains have been altered. We have consequently studied the temperature dependence of the base-on/base-off reaction of CNCbl (for which thermodynamic data did not previously exist), a series of seven alkyl-13-epicobalamins (R-13-epiCbl's[1]), in which epimerization at corrin ring C13 brings the *e* propionamide side chain to the upper corrin face, and a series of four alkyl-8-epicobalamins (R-8-epiCbl's[1]), in which epimerization at C8 makes the *d* propionamide side chain equatorial[28] (Figure 1). If a 5-coordinate/6-coordinate equilibrium is responsible for the trend in ΔS_{Co}, relocation of the *d* and *e* side chains should not affect the value of ΔS_{Co}. On the other hand, if side chain thermal motions and the effect of differential steric interactions with both axial ligands thereon are indeed important in the entropy changes associated with the off/on transition, relocation of the *d* or *e* side chain in the epicobalamins would be expected to significantly affect ΔS_{Co}. The results of these studies strongly suggest that differential corrin ring side chain thermal motions are not involved in the entropy change associated with the off/on reaction and have important consequences for understanding the thermodynamics of this intrinsic property of Cbl's, as described below.

Experimental Section

CNCbl was from Roussel Corp. The well-characterized[29] CN-13-epiCbl was prepared by acid-catalyzed epimerization of CNCbl in anhydrous trifluoroacetic acid[30,31] and purified as described previously.[22] CN-8-epiCbl was prepared by sodium borohydride reduction[32] of CNCbl-*c*-lactone,[33] followed by amidation of the *c*-monocarboxylate[34] as recently described.[28] This material has been rigorously characterized by complete assignment of its ^1H, ^{13}C, and amide ^{15}N NMR spectra, as well as determination of its X-ray crystal structure.[28]

R-13-epiCbl's (R = CH_3CH_2, $NCCH_2CH_2CH_2$, CH_3, CF_3CH_2, CF_2H, CF_3) and R-8-epiCbl's (R = CH_3CH_2, CF_3CH_2, CF_3) were obtained by standard reductive alkylation procedures as previously described.[10,12] Purity, as determined from HPLC chromatograms, was >95% throughout. These products were characterized by the UV–visible spectra of their base-on and base-off forms and by FAB MS (*m*-nitrobenzyl alcohol matrix[35,36]), which was carried out as previously described.[37] In every case, the observed parent ion in positive-ion FAB MS was within ±0.5 amu of that calculated for $M + H^+$, as is typically observed for zwitterionic RCbl's.[37,38] The UV–visible spectral properties and the calculated and observed FAB MS parent ion masses for the R-13-epiCbl's and R-8-epiCbl's are given in Table SI, available as supplementary material.

Values of $pK_{\text{base-off}}$ (eq 1) were determined by spectrophotometric titration at the visible wavelength of maximal spectral change, at ionic strength 1.0 M (adjusted with KCl), as previously described,[10] except those for CNCbl and CN-13-epiCbl. Absorbance data at various pH's at a given temperature were fitted to eq 7, where A_x is the absorbance

$$A_x = (K_{\text{base-off}}A_{A^-} + [H^+]A_{AH})/(K_{\text{base-off}} + [H^+]) \qquad (7)$$

at pH_x and A_{A^-} and A_{AH} are the base and acid end point absorbances, respectively, by a nonlinear, iterative least-squares method to determine $K_{\text{base-off}}$, A_{A^-}, and A_{AH}. For CNCbl, CN-13-epiCbl, and CN-8-epiCbl, the titrations were carried out in sulfuric acid/water mixtures using the generalized acidity function of Cox and Yates[39] (eq 8), where C_{H^+} is the

$$-H = m^*X + \log C_{H^+} \qquad (8)$$

concentration of hydrogen ion, X is the "excess acidity", and m^* is an adjustable parameter reflecting the solvation demands of the protonated species being studied and temperature-dependent corrections to the excess acidity function and $\log C_{H^+}$.[40] In this case, absorbance vs X, $\log C_{H^+}$ data sets were fitted simutaneously to eqs 7 and 8 to determine $K_{\text{base-off}}$, A_{AH}, A_{A^-}, and m^*. Values of m^* thus obtained varied from 0.72 to 1.02, but fitting any individual data set to eq 7 alone using fixed values of m^* showed that there is very little variation in $pK_{\text{base-off}}$ over this range of m^* values.

Values of $pK_{\text{base-off}}$ were determined for each compound at 5, 15, 25, and 35 °C and used to calculate K_{Co} (eq 3) from eq 4, using as K_{Bz} the values of the K_a of the conjugate acid of α-ribazole at the appropriate temperature.[10] For CH_3CH_2-13-epiCbl and CH_3CH_2-8-epiCbl, eq 6 was used to calculate K_{Co}, using the temperature-independent value of K_H (4.16) previously determined for CH_3Cbl.[12] Values of ΔH_{Co} and ΔS_{Co} were calculated from the slopes and intercepts, respectively, of plots of ln K_{Co} vs 1/T. The observed values of $pK_{\text{base-off}}$, A_{A^-}, A_{AH}, and the wavelength used in each individual titration are given in Table SII (supplementary material), and examples of spectrophotometric titration data are shown in Figures S1–S4 (supplementary material).

Results

Values of $pK_{\text{base-off}}$ determined at 25.0 ± 0.1 °C for CNCbl, the R-13-epiCbl's, the R-8-epiCbl's and those previously reported for the RCbl's[12] are given in Table 1, along with the calculated values of K_{Co} (eq 3). In Table 2, values of the enthalpy change, ΔH_{Co}, and the entropy change, ΔS_{Co}, for eq 3 are given for the same 21 complexes.

The value of $pK_{\text{base-off}}$ (0.03) reported here for CNCbl by spectrophotometric titration in H_2SO_4/H_2O mixtures is in reasonable agreement with those previously reported from observation of the ^{13}C resonance of $^{13}CNCbl$ (0.11)[41] and observation of the ^{15}N resonance of $C^{15}NCbl$ (0.09)[42] in similar media. However, both ΔH_{Co} and ΔS_{Co} fail to follow the trends seen for the other RCbl's. As previously reported[12] for the RCbl's, where R ≠ CN, ΔH_{Co} is largely independent of R, with an average value of -7.8 ± 0.6 kcal mol^{-1}. For CNCbl ($\Delta H_{Co} = -9.1 \pm 0.3$ kcal mol^{-1}), the reaction is clearly significantly more exothermic. Similarly, the gradual decline of $-\Delta S_{Co}$ across the series of RCbl's halts with CNCbl, for which ΔS_{Co} is essentially identical to that for CF_3Cbl, despite the fact that K_{Co} is 25-fold higher for CNCbl than for CF_3Cbl. Thus, with the extension of the thermodynamic data for RCbl's to CNCbl, the downward trend of $-\Delta S_{Co}$ is seen to level off at high values of K_{Co}, while ΔH_{Co}, essentially constant when R ≠ CN, becomes more negative.

Epimerization at C13, which removes the *e* propionamide side chain from the lower corrin face, causes a decrease in $pK_{\text{base-off}}$, which averages 0.83 ± 0.14 across the series, and a corresponding increase in K_{Co}, for a given R. On average, K_{Co} is (6.9 ± 1.8)-fold higher for the R-13-epiCbl's than for the RCbl's, corresponding to an average $\Delta\Delta G_{Co}$ of -1.1 ± 0.4 kcal mol^{-1}. Most importantly, however, this difference in free energy is entirely due to differences in ΔH_{Co}, the values of ΔS_{Co} being experimentally indistinguishable for a given R-13-epiCbl and its RCbl counterpart, for all six pairs of compounds observed. Thus, the average difference in the enthalpy change for Bzm coordination, $\Delta\Delta H_{Co} = -1.1 \pm 0.6$ kcal mol^{-1}, completely accounts for the average difference in free energy

---

(28) Brown, K. L.; Zou, X.; Wu, G.-Z.; Zubkowski, J. D.; Valente, E. J. Submitted for publication in *Inorg. Chem.*

(29) Stoeckli-Evans, H.; Edmond, E.; Hodgkin, D. C. *J. Chem. Soc., Perkin Trans. 2* **1972**, 602.

(30) Bonnett, R.; Godfrey, J. M.; Math, V. B.; Edmond, E.; Evans, H.; Hodder, O. J. R. *Nature* **1971**, *229*, 473.

(31) Bonnett, R.; Godfrey, J. M.; Math, V. B. *J. Chem. Soc. C* **1971**, 3736.

(32) Rapp, P.; Oltersdorf, U. *Hoppe-Seyler's Z. Physiol. Chem.* **1973**, *354*, 32.

(33) Bonnett, R.; Cannon, J. R.; Johnson, A. W.; Parker, L. F. J.; Smith, E. L.; Todd, A. *J. Chem. Soc.* **1957**, 1158.

(34) Toraya, T.; Krodel, E.; Mildvan, A. S.; Abeles, R. H. *Biochemistry* **1979**, *18*, 417.

(35) Meili, J.; Seibl, *J. Org. Mass Spectrom.* **1984**, *21*, 793.

(36) Sharp, T. R.; Santander, P. J.; Scott, A. I. *Tetrahedron Lett.* **1990**, *43*, 6163.

(37) Brown, K. L.; Salmon, L.; Kirby, J. A. *Organometallics* **1992**, *11*, 422.

(38) Brown, K. L.; Evans, D. R. *Inorg. Chem.* **1990**, *29*, 2559.

(39) Cox, R. P.; Yates, K. *J. Am. Chem. Soc.* **1978**, *100*, 3861.

(40) Cox, R. A.; Goldman, M. F.; Yates, K. *Can. J. Chem.* **1979**, *57*, 2960.

(41) Brown, K. L.; Hakimi, J. M. *Inorg. Chem.* **1984**, *23*, 1756.

(42) Brown, K. L.; Gupta, B. D. *Inorg. Chem.* **1990**, *29*, 3854.

Table 1. Values of $pK_{base-off}$ and K_{Co} for RCbl's, R-13-epiCbl's, and R-8-epiCbl's at 25.0 °C[a]

R	RCbl[b]		R-13-epiCbl		R-8-epiCbl	
	$pK_{base-off}$	K_{Co}	$pK_{base-off}$	K_{Co}	$pK_{base-off}$	K_{Co}
CH_3CH_2	4.16 ± 0.01	(2.00 ± 0.11) × 10	3.51 ± 0.02	(1.07 ± 0.07) × 10^2	4.62 ± 0.02	3.52 ± 0.43
$CH_3(CH_2)_2$	4.10 ± 0.01	(2.37 ± 0.13) × 10				
$NC(CH_2)_3$	3.50 ± 0.01	(1.10 ± 0.05) × 10^2	2.81 ± 0.01	(5.57 ± 0.26) × 10^2		
$CH_3OOC(CH_2)_2$	3.33 ± 0.01	(1.65 ± 0.08) × 10^2				
CH_3	2.90 ± 0.01	(4.52 ± 0.21) × 10^2	2.15 ± 0.02	(2.55 ± 0.18) × 10^3		
CF_3CH_2	2.60 ± 0.01	(9.23 ± 0.42) × 10^2	1.82 ± 0.02	(5.44 ± 0.38) × 10^3	3.08 ± 0.01	(2.98 ± 0.14) × 10^2
CF_2H	2.15 ± 0.01	(2.60 ± 0.12) × 10^3				
$NCCH_2$	1.81 ± 0.01	(5.62 ± 0.26) × 10^3	0.85 ± 0.01	(5.14 ± 0.24) × 10^4		
CF_3	1.44 ± 0.01	(1.32 ± 0.06) × 10^4	0.47 ± 0.03	(1.13 ± 0.10) × 10^5	1.75 ± 0.01	(6.51 ± 0.30) × 10^3
CN	0.03 ± 0.03[c]	(3.35 ± 0.31) × 10^5 [c]	−0.90 ± 0.03	(2.88 ± 0.27) × 10^6	0.50 ± 0.02	(1.14 ± 0.07) × 10^5

[a] Ionic strength 1.0 M, except for CNCbl and CN-13-epiCbl. See Experimental Section. [b] Reference 12, except as noted. [c] This work.

Table 2. Enthalpies and Entropies for the Base-On/Base-Off Reaction of RCbl's, R-13-epiCbl's, and R-8-epiCbl's[a]

R	RCbl[b]		R-13-epiCbl		R-8-epiCbl	
	ΔH_{Co} (kcal mol^{-1})	ΔS_{Co} (cal mol^{-1} K^{-1})	ΔH_{Co} (kcal mol^{-1})	ΔS_{Co} (cal mol^{-1} K^{-1})	ΔH_{Co} (kcal mol^{-1})	ΔS_{Co} (cal mol^{-1} K^{-1})
CH_3CH_2	−8.8 ± 0.7	−24 ± 3	−8.9 ± 0.4	−21 ± 1	−7.1 ± 0.5	−22 ± 2
$CH_3(CH_2)_2$	−8.3 ± 0.8	−22 ± 3				
$NC(CH_2)_3$	−7.9 ± 1.8	−17 ± 2	−8.6 ± 0.4	−16 ± 1		
$CH_3OOC(CH_2)_2$	−8.0 ± 0.4	−17 ± 2				
CH_3	−7.6 ± 0.4	−13 ± 1	−9.4 ± 0.4	−16 ± 1		
CF_3CH_2	−8.0 ± 0.7	−14 ± 2	−9.0 ± 0.3	−13 ± 1	−7.6 ± 0.8	−14 ± 1
CF_2H	−6.8 ± 0.5	−7.3 ± 1.5				
$NCCH_2$			−8.4 ± 0.2	−6.5 ± 0.6		
CF_3	−7.0 ± 0.3	−4.7 ± 1.2	−8.5 ± 0.6	−5.3 ± 2.3	−6.5 ± 0.1	−4.5 ± 0.3
CN	−9.1 ± 0.3[c]	−5.1 ± 1.1[c]	−10.6 ± 0.3	−6.1 ± 1.1	−7.8 ± 0.5	−3.0 ± 1.8

[a] Equation 3. [b] Reference 12, except as noted. [c] This work.

change. It is possible that, in the RCbl's, the downward projecting *e* propionamide side chain interferes with Bzm coordination, lowering the binding free energy by about 1 kcal mol^{-1}. However, there are differences in the inner-sphere geometry between CNCbl and CN-13-epiCbl,[27,29] and recent results[43] show significant ^{13}C chemical shift differences in the organic ligand of neopentyl-Cbi$^+$ and neopentyl-13-epiCbi$^+$. Thus, electronic differences at the cobalt atom may be responsible for the difference in ΔG_{Co} for RCbl's and R-13-epiCbl's.

Across the series of seven R-13-epiCbl's, the trends in ΔH_{Co} and ΔS_{Co} can be seen to mimic those in the RCbl series (Table 2). Thus, with the exception of that for CN-13-epiCbl, ΔH_{Co} does not depend on R, the average value being −8.8 ± 0.4 kcal mol^{-1}. However, for R = CN, there is a significant increase in exothermicity (ΔH_{Co} = −10.6 ± 0.3 kcal mol^{-1}), as shown graphically in Figure 2A, a plot of $-\Delta H_{Co}$ vs $-\Delta G_{Co}$ for the R-13-epiCbl's. In the same vein, the trend of a gradual decrease in $-\Delta S_{Co}$ with increasing affinity of the Bzm ligand for the metal atom levels off with a limiting value of $\Delta S_{Co} \approx -6$ cal mol^{-1} K^{-1}. This effect is also shown graphically in Figure 2B, a plot of $-\Delta S_{Co}$ vs $-\Delta G_{Co}$ for the R-13-epiCbl's.

For the R-8-epiCbl's, in which the *d* propionamide side chain is removed from the corrin ring lower face, the values of $pK_{base-off}$ are higher than those for the RCbl's by an average of 0.43 ± 0.08. This corresponds to a (3.4 ± 1.6)-fold decrease in K_{Co}, or an average $\Delta\Delta G_{Co}$ of 0.7 ± 0.2 kcal mol^{-1}. Again, however, this difference is entirely due to the differences in ΔH_{Co}, as the values of ΔS_{Co} for the R-8-epiCbl's are experimentally indistinguishable from those for the RCbl's and the R-13-epiCbl's for a given R. Thus, as was the case for the R-13-epiCbl's, the difference between the enthalpy change for the R-8-epiCbl's and the analogous RCbl's, $\Delta\Delta H_{Co}$ = 1.0 ± 0.6 kcal mol^{-1}, accounts for all of the difference in the free energy changes. For the R-8-epiCbl's, the loss of ≈0.75 kcal of free energy for the coordination of the Bzm nucleotide upon removal of the *d* propionamide from the lower corrin face can be seen as the result of the loss of a hydrogen-bonding interaction between the *d* side chain amide and the Bzm B1 nitrogen (Figure 1) in base-on RCbl's previously observed by ^1H and ^{15}N NMR studies.[44] Evidently, this hydrogen bond stabilizes the base-on species of RCbl's by about 0.75 kcal, an appropriate value for the free energy of a hydrogen bond between an uncharged donor and acceptor in water.[45]

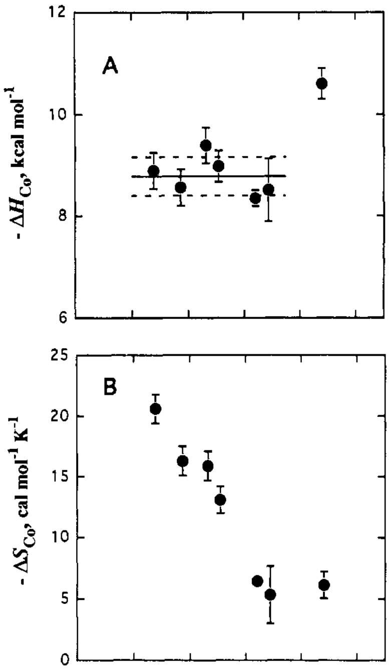


Figure 2. (A) Plot of $-\Delta H_{Co}$ vs $-\Delta G_{Co}$ for the base-off/base-on equilibrium of the R-13-epiCbl's (eq 3). The vertical bars represent plus or minus one standard deviation. The solid line is the average of the $-\Delta H_{Co}$ values for all R ≠ CN, and the dashed lines are plus or minus one standard deviation. (B) Plot of $-\Delta S_{Co}$ vs $-\Delta G_{Co}$ for the base-off/base-on equilibrium of the R-13-epiCbl's (eq 3). The vertical bars represent plus or minus one standard deviation and are included in the symbol where not shown.

---

(43) Brown, K. L.; Evans, D. R. Submitted for publication in *Magn. Reson. Chem.*

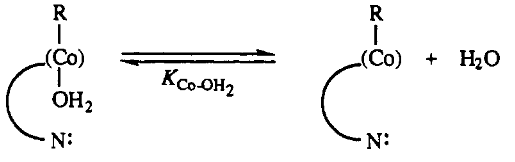
(44) Brown, K. L.; Evans, D. R. *Inorg. Chem.* **1993**, *32*, 2544.

Although the series of R-8-epiCbl complexes is less extensive than those of the RCbl's and R-13-epiCbl's, the trends in ΔH_{Co} and ΔS_{Co} are clearly similar. Thus, the value of ΔH_{Co} for the R-8-epiCbl's is relatively constant for R ≠ CN, although the average value, −7.1 ± 0.5 kcal mol^{-1}, is not significantly different from the value for CN-8-epiCbl (−7.8 ± 0.5 kcal mol^{-1}). As was the case for the RCbl's and the R-13-epiCbl's, $-\Delta S_{Co}$ decreases significantly across the series but levels off at R = CN, the values of ΔS_{Co} for CF_3-8-epiCbl and CN-8-epiCbl being experimentally indistinguishable.

Discussion

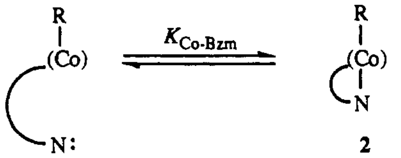
The data in Table 2 effectively demonstrate that the entropy change associated with the base-off/base-on reaction of eq 3 is not significantly affected by epimerization at C8 or at C13. Thus, although these epimerizations significantly affect $pK_{base-off}$ and K_{Co}, in every case the effect is entirely in the enthalpy change, ΔH_{Co}. Since these structural modifications change both the number and type of side chains at the lower and upper corrin ring faces, the lack of dependence of the entropy change for formation of the base-on species, **2**, upon RCbl epimerization strongly suggests that differential side chain thermal motions are not a significant contributor to the entropy changes accompanying the off/on transition. If this is indeed the case, then ΔS_{Co} must be dominated by the entropic effects of the changes in axial ligation since no other part of the molecule possesses sufficient internal freedom of motion to contribute significantly to the observed entropy changes.

For consideration of the entropic effects of the axial ligand changes accompanying the off/on transition, it is convenient to factor eq 3 into its ligand loss and ligand gain components, eqs 9–12. The entropy changes associated with eqs 9 and 11 must again be expected to be dominated by the effects of the loss of the water ligand and the coordination of the axial nucleotide, respectively, and be largely independent of the nature of R. While R may have some effect on the conformations of the various species and on their conformational mobility, the absence of an effect of side chain mobility on the overall entropy change argues that such effects will be small, since the side chains are by far the most flexible parts of these molecules.



3 5 (9)

$$K_{Co\text{–}OH_2} = [3]/[5] \tag{10}$$



5 2 (11)

$$K_{Co\text{–}Bzm} = [2]/[5] \tag{12}$$

If the off/on transition represented by eq 3 is always the simple sum of eqs 9 and 11, regardless of R, then the net entropy change, $\Delta S_{Co} = \Delta S_{Co\text{–}Bzm} - \Delta S_{Co\text{–}OH_2}$, would be expected to be largely independent of R, following the discussion above. However, the data show that this is not the case. In each series of compounds, ΔS_{Co} is strongly dependent on R and, in fact, is responsible for all of the observed variation in K_{Co} (except for R = CN; vide infra). This suggests that for the compounds with the most electron-donating R groups (R = CH_3CH_2, $CH_3CH_2CH_2$) and the most negative ΔS_{Co} values, the entropy loss associated with the restriction of the pendent axial nucleotide during formation of the base-on species (eq 11) is largely uncompensated for by the gain in entropy due to loss of an axial water ligand (eq 9): i.e., the base-off species of these complexes are largely pentacoordinate under these conditions. The dependence of the value of ΔS_{Co} on the nature of R can then be seen as being due to the dependence of $K_{Co\text{–}OH_2}$ (eqs 9 and 10) on R, since the net entropy change for formation of the base-on species (**2**) is given by eq 13,

$$\Delta S_{Co} = \Delta S_{Co\text{–}Bzm} - \alpha_{Co\text{–}OH_2}\Delta S_{Co\text{–}OH_2} \tag{13}$$

$$\alpha_{Co\text{–}OH_2} = [3]/([3] + [5]) \tag{14}$$

$$\alpha_{Co\text{–}OH_2} = K_{Co\text{–}OH_2}/(K_{Co\text{–}OH_2} + 1) \tag{15}$$

where $\alpha_{Co\text{–}OH_2}$ represents the fraction of the base-off species present as the hexacoordinate aqua complex (**3**), as in eqs 14 and 15. Thus, as the equilibrium of eq 9 is displaced to the left by increasingly electron-withdrawing R groups, the loss of entropy associated with axial Bzm coordination (eq 11) is compensated for by an increasing fraction of the entropy gain due to axial water loss. Eventually a point should be reached where $\alpha_{Co\text{–}OH_2} \approx 1$ and further increases in electron withdrawal by R can no longer lead to increases in $\alpha_{Co\text{–}OH_2}$. At this point, the net entropy change is given by $\Delta S_{Co} = \Delta S_{Co\text{–}Bzm} - \Delta S_{Co\text{–}OH_2}$ and no longer shows a significant dependence on R, as discussed above. This is precisely what is seen in the ΔS_{Co} data (Table 2 and Figure 2B), the point at which $\alpha_{Co\text{–}OH_2} \approx 1$ apparently being reached when R = CF_3. These data suggest that the net difference in entropy, $\Delta S_{Co\text{–}Bzm} - \Delta S_{Co\text{–}OH_2}$, is about −4 to −5 cal mol^{-1} K^{-1} (the average value of ΔS_{Co} for R = CF_3 and CN in all three epimers is −4.8 ± 1.0 cal mol^{-1} K^{-1}), i.e., that the loss of entropy due to restriction of the nucleotide loop by Bzm coordination is about 4–5 eu larger than the gain of entropy due to dissociation of the axial water ligand.

This model also provides a more satisfying explanation for the apparent lack of dependence of ΔH_{Co} on R, for R ≠ CN, than the one previously offered, i.e., that both the Co–OH_2 and Co–N_{Bzm} bond enthalpies have the same dependence on the nature of R. Thus, the net enthalpy change for formation of the base-on species, **2**, is given by eq 16, analogous to eq 13. Changes in

$$\Delta H_{Co} = \Delta H_{Co\text{–}Bzm} - \alpha_{Co\text{–}OH_2}\Delta H_{Co\text{–}OH_2} \tag{16}$$

$\Delta H_{Co\text{–}Bzm}$ are thus increasingly compensated for by an ever larger fraction of $\Delta H_{Co\text{–}OH_2}$ as we progress to more electron-withdrawing R through the series. If the Co–N_{Bzm} bond enthalpy were indeed more sensitive to R than the Co–OH_2 bond enthalpy, this would produce a leveling effect that could lead to the apparent R independence of ΔH_{Co}, as long as $\alpha_{Co\text{–}OH_2}$ continues to increase. When the point is reached where $\alpha_{Co\text{–}OH_2} \approx 1$, no further leveling would occur, and ΔH_{Co} would be expected to increase with increasing electron withdrawal by R. This again is what is observed in each of the series, the value of ΔH_{Co} for R = CN being 1.3 ± 0.7, 1.8 ± 0.5, and 0.8 ± 0.7 kcal mol^{-1} more negative than the average value for the other complexes in the RCbl, R-13-epiCbl, and R-8-epiCbl series, respectively.

The possibility that the base-off species of RCbl's and the analogous alkylcobinamides ($RCbi^+$),[1] in which the axial nucleotide has been removed by phosphodiester hydrolysis (Figure 1), might exist as mixtures of pentacoordinate and hexacoordinate

---

(45) Fersht, A. R.; Shi, J. P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Waye, M. M. Y.; Winter, G. *Nature* **1985**, *314*, 235.

aquo species was first raised by Pratt and co-workers.[46–49] These authors attributed temperature-dependent UV–visible spectral changes and the temperature dependence of the ^1H NMR chemical shift of the C10 hydrogen of such complexes to the existence of pentacoordinate/hexacoordinate equilibrium and found the equilibrium to be sensitive to the nature of the organic ligand. Similar indirect evidence of pentacoordinate alkylcobalt species has been reported for alkylcobaloximes[1,50,51] and alkylcobalt octaethylporphyrins,[52] as well as alkylcobalt complexes in other equatorial ligand systems.[53] Indeed, X-ray crystal structures of $((CH_3)_2CH)Co(Saloph)$,[1,54] $CH_3Co(Saloph)$,[1,54] and $CH_3Co(BAE)$[1,55] have shown that these materials are pentacoordinate in the solid state. In addition, $RCbi^+$ complexes are known to be pentacoordinate in the gas phase under fast atom bombardment, as shown by their FAB mass spectra.[37,38,56,57] Recently, Wirt and Chance[58] provided evidence of a pentacoordinate/hexacoordinate equilibrium in base-off CH_3Cbl and AdoCbl from the temperature dependence of the 1s–3d pre-edge transition in the X-ray absorption edge spectra of these complexes.

Thus, spectroscopic evidences of various kinds are available for the existence of a pentacoordinate/hexacoordinate equilibrium for alkyl(aqua)cobalt corrinoids and for its sensitivity to the inductive effect of the alkyl ligand. To these data we now add the thermodynamic results presented here that strongly suggest such an equilibrium for the base-off RCbl's and their epimers and suggest that this equilibrium is displaced toward the hexacoordinate aquo species as the organic ligand becomes more electron withdrawing. Further evidence for such coordination dynamics in organocobalt corrinoids is currently being sought from magnetic circular dichroism measurements.

Conclusion

Epimerization of alkylcobalamins at C8 or C13, which moves the *d* or *e* propionamide side chain, respectively, from the lower corrin face to the upper corrin face, has been found to raise or lower the free energy of formation of the base-on species from the unprotonated base-off species, by about 1 or 0.75 kcal mol^{-1}, respectively. However, the effect is expressed in the enthalpy change for ligand substitution, the entropy change being independent of the epimer for a given alkyl group. These results suggest that changes in corrin ring side chain thermal motions do not significantly influence the entropy changes for the base-off/base-on reaction, which, instead, must be dominated by the entropic effects of the entering and leaving ligands. For each of the epimeric alkylcobalt corrinoids, there is a large decrease in $-\Delta S$ for this ligand exchange process across a series of compounds in which the organic ligand is increasingly electron withdrawing and $-\Delta G$ progressively increases. For compounds with the most electron-withdrawing groups and the largest values of $-\Delta G$, the entropy change becomes independent of the organic ligand and has a value of ca. −4 to −5 cal mol^{-1} K^{-1}. This behavior is explained if the base-off species of these compounds undergo a pentacoordinate/hexacoordinate equilibrium, the position of which is sensitive to the inductive effect of the organic ligand, such that, for the compounds with the highest value of $-\Delta S$ (and the lowest $-\Delta G$), the loss of entropy upon coordination of the pendent axial nucleotide is largely uncompensated for by the entropic effect of the loss of an axial water ligand: i.e., these compounds are largely pentacoordinate. As the organic ligand becomes more inductively withdrawing, the pentacoordinate/hexacoordinate equilibrium of the base-off species shifts toward the hexacoordinate aquo species and there is increasing compensation for the entropy loss from the entropy gain of H_2O dissociation. Toward the end of the series of compounds, the base-off species is completely hexacoordinate and the net entropy change no longer varies with the alkyl ligand. In a similar fashion, gradually increasing enthalpic compensation due to H_2O loss across the series of compounds leads to a leveling of the net enthalpy change, such that the net enthalpy of formation of the base-on species is independent of the organic ligand for most of the series but becomes more negative once the base-off species is fully hexacoordinate.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

Supplementary Material Available: Table SI, giving the UV–visible and FAB mass spectral data for the R-13-epiCbl and R-8-epiCbl complexes, Table SII, giving the results of the individual titrations of each complex at each of four temperatures, and Figures S1–S4, showing sample titration data (10 pages). Ordering information is given on any current masthead page.

---

(46) Firth, R. A.; Hill, H. A. O.; Mann, B. E.; Pratt, J. M.; Thorp, R. G. *Chem. Commun.* **1967**, 1013.
(47) Firth, R. A.; Hill, H. A. O.; Mann, B. E.; Pratt, J. M.; Thorp, R. G.; Williams, R. J. P. *J. Chem. Soc. A* **1968**, 2419.
(48) Hill, H. A. O.; Pratt, J. M.; Williams, R. J. P. *Discuss. Faraday Soc.* **1969**, *47*, 165.
(49) Chamaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2259.
(50) Brown, K. L.; Lyles, D.; Pencovici, M.; Kallen, R. G. *J. Am. Chem. Soc.* **1975**, *97*, 7338.
(51) Brown, K. L.; Awtrey, A. W. *Inorg. Chem.* **1978**, *17*, 111.
(52) Ogashi, H.; Watanabe, E.; Koketsu, N.; Yoshida, Z. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2829.
(53) See ref 46 and references therein.
(54) Marzilli, L. G.; Summers, M. F.; Bresciani-Pahor, N.; Zangrando, E.; Charland, J. P.; Randaccio, L. *J. Am. Chem. Soc.* **1985**, *107*, 6880.
(55) Buckner, S.; Calligaris, M.; Nardin, G.; Randaccio, L. *Inorg. Chim. Acta* **1969**, *3*, 308.
(56) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012.
(57) Brown, K. L.; Zou, X. *J. Am. Chem. Soc.* **1992**, *114*, 9643.
(58) Wirt, M. D.; Chance, M. R. *J. Inorg. Biochem.* **1993**, *49*, 265.